

Patent Application
Docket No. SPO.129

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner : Philip Dubois
Art Unit : 1781
Applicants : Hisae Kume *et al.*
Serial No. : 10/593,550
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Conf. No. : 4478
For : Antibacterial Compositions

Commissioner for Patents
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SECOND DECLARATION OF HISAE KUME UNDER 37 CFR §1.132

Sir:

I, Hisae Kume, hereby declare:

THAT, I am a Research Scientist;

THAT, my qualifications are set forth in more detail in the copy of my Statement of Qualifications attached to my earlier Declaration filed November 1, 2010 and of record in this application;

THAT, I am an inventor on the above-referenced application;

THAT, I, have reviewed the specification, the pending claims, the references cited, and the Office Action mailed December 27, 2010;

And being thus duly qualified, do further declare as follows:

Our invention provides a novel antibacterial composition that inhibits the proliferation of Gram-positive bacteria. The antibacterial composition is prepared by mixing a fermented dairy product with carbohydrates, proteins and fats. The antibacterial composition has a pH of 4.6 or less, and the energy ratio of carbohydrates, proteins, and fats contained therein is 50% to 70%, 4% to 25%, and 20% to 30%, respectively.

In this Declaration, I report results of a comparative experiment, which compares the antibacterial activity of the acidic liquid diet of our invention to various control compositions, including a neutral liquid diet, LA lactic acid bacteria beverage (banana flavor), LA lactic acid bacteria beverage (berry flavor), fermented milk preparation, and Bulgaria Yogurt (Meiji Dairies Corporation).

The results, as shown in Figure 1, demonstrate that the acidic liquid diet of our invention has unexpectedly superior antibacterial effects against Gram-positive bacteria, when compared to the neutral liquid diet, the lactic acid bacteria beverages (banana flavor and berry flavor), the fermented milk preparation, and the Bulgaria Yogurt (Meiji Dairies Corporation).

Materials and Method

1. Outline of the Experiment (commissioned to the Japan Food Research Laboratories)

The samples were prepared by adding a bacterial solution of *Staphylococcus aureus* IID 1677 (methicillin-resistant *Staphylococcus aureus*: MRSA) to each of the compositions (100 mL) listed below, and mixing the bacterial solution with the composition. The samples were incubated at 37°C, and viable bacterial cell counts were determined 3, 6, and 24 hours later.

2. Compositions

The compositions used in the experiment include:

- (1) neutral liquid diet (Meibalance), pH6.71 (without a fermented dairy product);
- (2) acidic liquid diet (Fibren YH), pH4.16 (including a fermented dairy product);
- (3) *Lactobacillus acidophilus*-5 banana flavor (imported from Latteria Sociate Merano Soc. Agricola Coop., Italy), pH4.07 (LA lactic acid bacteria beverage: banana flavor);
- (4) *Lactobacillus acidophilus*-5 berry flavor, pH4.01 (LA lactic acid bacteria beverage: berry flavor);
- (5) fermented milk preparation (quark), prepared by dissolving quark in water and

adjusting the final concentration of quark to 40 g/100 mL (the same amount of quark was used to prepare the fermented milk preparation and the acidic liquid diet), pH4.07; and

(6) Bulgaria Yogurt (Meiji Dairies Corporation; milk was fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*), pH4.37.

Compositions (1) and (2) are manufactured products and, thus, had been sterilized. Compositions (3) to (6) were sterilized by autoclave treatment at 95°C for 5 minutes.

Results

Figure 1 shows the antibacterial action of the compositions against MRSA. The acidic liquid diet of our invention has unexpectedly superior antibacterial activity against Gram-positive bacteria, as the bacterial count of MRSA detected in the 24-hour culture was 10 cfu/g or less, showing that the bacterial count was lower than the detection limit.

In comparison, the neutral liquid diet (Meibalance) has little or no anti-MRSA activity. The lactic acid bacteria beverages (Compositions (3) and (4)) and the fermented milk products (Composition (5) and (6)) have similar anti-MRSA activity, which are significantly weaker than the anti-MRSA activity of the acidic liquid diet of our invention.

Table 1 shows the energy ratios of carbohydrates, proteins, and fats of the LA lactic acid bacteria beverages, the acidic liquid diet, and the neutral liquid diet. As shown in Table 1, the energy ratios of carbohydrates, proteins, and fats of the LA lactic acid bacteria beverages are outside of the scope of the claims of the current patent application.

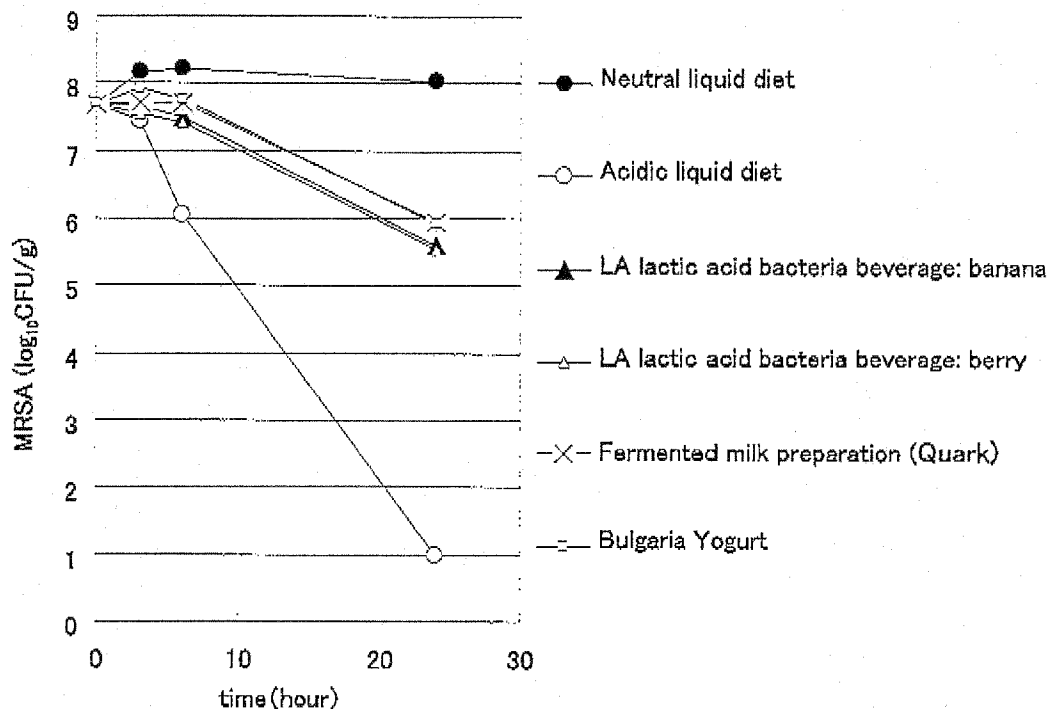
The energy ratios of carbohydrates, proteins, and fats of the neutral liquid diet are within the ranges recited in the claims of the current patent application. However, the neutral liquid diet differs from the acidic liquid diet of our invention in that (i) a fermented dairy product is not used, and (ii) the pH value is higher than 4.6.

Table 1 Energy ratios of carbohydrates, proteins, and fats of the LA lactic acid bacteria beverages, the acidic liquid diet, and the neutral liquid diet.

| | LA lactic acid bacteria beverage | | Acidic liquid diet | | Neutral liquid diet | |
|---------------|-------------------------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| | contents (wt%) | energy ratio(%) | contents (wt%) | energy ratio(%) | contents (wt%) | energy ratio(%) |
| proteins | 2.9 | 14.8 | 4 | 16.4 | 4 | 16.1 |
| fats | 1.5 | 17.2 | 2.8 | 25.8 | 2.8 | 25.4 |
| carbohydrates | 13.3 | 67.9 | 14.1 | 57.8 | 14.5 | 58.5 |

The results also show that a fermented dairy product included in, e.g., the LA lactic acid bacteria beverages, fermented milk preparation (quark), or Bulgaria Yogurt is not the sole or sufficient factor for antibacterial activity against Gram-positive bacteria. Rather, the unexpectedly superior antibacterial activity against Gram-positive bacteria is achieved by a synergistic action among the fermented dairy product, the pH value of 4.6 or less and the claimed energy ratio of carbohydrates, proteins and fats of 50% to 70%, 4% to 25%, and 20% to 30%, respectively.

Figure 1 Antibacterial Effects against MRSA



Conclusion

The acidic liquid diet of our invention has unexpectedly superior antibacterial activity against Gram-positive bacteria, when compared to the neutral liquid diet, the lactic acid bacteria beverages, the fermented milk preparation, and the Bulgaria Yogurt (Meiji Dairies Corporation). This unexpectedly superior antibacterial activity against Gram-positive bacteria is achieved by a

5

Docket No. SPO.129
Serial No. 10/593,550

synergistic action among the fermented dairy product, the pH value of 4.6 or less and the energy ratio of carbohydrates, proteins and fats of 50% to 70%, 4% to 25%, and 20% to 30%, respectively.

I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: June 20, 2011

Hisae Kume

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